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Effects of Hypothermia on the In vivo Measurement of Rapid Axonal Transport in the Rat: A Cautionary Note

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Rapid axonal transport of glycoproteins was examined in the retinofugal projections of hypothermic and normothermic adult male Long-Evans hooded rats previously receiving intraocular injections of [^{3}H]fucose. The amount of retinal fucosylation appeared normal in the hypothermic animals 3.5 h after isotope injection, but glycoprotein transport was reduced relative to normothermic controls. This reduction was especially pronounced in the most distal structure of the retinofugal tract (superior colliculus). We conclude that rapid axonal transport decreases with reductions in mammalian body temperature. This finding emphasizes the importance of controlling body temperature in in vivo studies of mammalian axonal transport. Key Words: Axonal transport—Fucose—Rat optic system—Hypothermia—Glycoproteins. Padilla S. and Lyerly D. Effects of hypothermia on the in vivo measurement of rapid axonal transport in the rat: A cautionary note. *J. Neurochem.* 46, 1227–1230 (1986).

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Effects of Hypothermia on the In Vivo Measurement of Rapid Axonal Transport in the Rat: A Cautionary Note

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Abstract: Rapid axonal transport of glycoproteins was examined in the retinofugal projections of hypothermic and normothermic adult male Long-Evans hooded rats previously receiving intraocular injections of [^{3}H]fucose. The amount of retinal fucosylation appeared normal in the hypothermic animals 3.5 h after isotope injection, but glycoprotein transport was reduced relative to normothermic controls. This reduction was especially pronounced in the most distal structure of the retinofugal tract (superior colliculus). We conclude that rapid axonal transport decreases with reductions in mammalian body temperature. This finding emphasizes the importance of controlling body temperature in in vivo studies of mammalian axonal transport. **Key Words:** Axonal transport—Fucose—Rat optic system—Hypothermia—Glycoproteins. **Padilla S. and Lyerly D.** Effects of hypothermia on the in vivo measurement of rapid axonal transport in the rat: A cautionary note. *J. Neurochem.* **46**, 1227–1230 (1986).

Although mammals normally maintain body temperature within a small, constant range, disruptions of their thermoregulatory physiology may convert them into poikilotherms, inducing either hyperthermia or hypothermia depending on the ambient temperature (Cremer and Bligh, 1969). These poikilothermic animals may exhibit hypothermia in a normal laboratory environment, because that temperature may be considerably below their isothermic temperature. Substantial biochemical differences exist between normothermic and hypothermic mammals (Cremer, 1967, 1971); differences that may not be a direct result of the treatment, but rather an indirect result due to treatment-induced hypothermia (Shuster and Hannam, 1964; Rose and Aldridge, 1966). Of the neurochemical parameters that could be affected by hypothermia, axonal transport has been demonstrated to be temperature-dependent using in vitro and in vivo preparations in poikilotherms (e.g., Elam and Agranoff, 1971; Grafstein et al., 1972; Heslop and Howes, 1972; Edström and Hanson, 1973; Gross, 1973; Cancalon, 1979) and using in vitro preparations in mammals (e.g., Ochs and Smith, 1975; Cosens et al., 1976; Bisby and Jones, 1978; Kim and Johnson, 1982). To date, several studies have had conflicting results when measuring axonal transport profiles in drug- or toxicant-treated animals or in mammals with metabolic disorders possibly because of inadequate controls for differences in body temperature. However, some recent studies commenting on the in vivo measurement of mammalian axonal transport have taken into account the possible confounding effects of body temperature (Stromska and Ochs, 1982; Tomlinson and James, 1984; Boyes et al., 1985). The present experiment was undertaken to delineate the extent of this hypothermic effect on the in vivo measurement of rapid axonal transport in rats using Chloropent anesthesia to render the animals poikilothermic so that body temperature could be controlled by manipulation of ambient temperature.

MATERIALS AND METHODS

Long-Evans male rats (427.3 ± 12.5 g, mean ± SEM, Charles River Breeding, Portage, ME, U.S.A.) were in-

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Abbreviations used: PTA, phosphotungstic acid; TCA, trichloroacetic acid.

jected intraperitoneally with an anesthetic dosage (0.37 ml/100 g body weight) of Chloropent (Fort Dodge Lab, Fort Dodge, IA, U.S.A.). Composition of Chloropent per milliliter was: chloral hydrate, 42.5 mg; magnesium sulfate, 21.2 mg; pentobarbital, 8.86 mg; ethyl alcohol, 14.25%; propylene glycol, 33.8%; distilled water, q.s. The Chloropent-treated animals were divided randomly into two groups. One group was placed in a water-lined incubator (model DW-3, Thermocare, Incline Village, NV, U.S.A.) maintained at a temperature of 31.6 ± 0.4°C. The other group was placed in a second incubator maintained at 22.8 ± 0.3°C. To prolong the effects of Chloropent, the animals received a second injection of Chloropent (subcutaneously, 0.185 ml/100 g body weight) 1.5 h after the first injection. Control animals received equivalent volumes of saline in place of all Chloropent injections, were not placed in incubators, but remained at room temperature (21°C). Sixty minutes after the injection of Chloropent or saline, the left eye of each animal was injected with 14–21 μCi [^{3}H]fucose in 0.5 μl saline (L-[6-^{3}H]fucose, 10–15 Ci/mmol, New England Nuclear, Boston, MA, U.S.A.). The rectal temperature of each animal was monitored during the experiment using a Yellow Springs thermometer (Yellow Springs Instrument, Yellow Springs, OH, U.S.A.).

The animals were decapitated 3.5 h after the isotope injection, and the right and left retinas, optic nerves, optic tracts, lateral geniculate bodies, and superior colliculi were removed and placed in an ice-cold solution of 10% trichloroacetic acid/0.5% phosphotungstic acid (TCA/PTA). Only the 5 mm length of optic nerve immediately proximal to the optic chiasm was used as a sample of optic nerve to lessen the possibility of measuring [^{3}H]fucose that arrived by diffusion rather than axonal transport (Haley et al., 1979).

In each tissue sample, glycoproteins were TCA/PTA precipitated, washed (Rapaport and Benjamins, 1981) using Gelman glass filters (Type A-E, Gelman Sciences, Ann-Arbor, MI, U.S.A.), digested in 2.0 ml Protosol (New England Nuclear, Boston, MA, U.S.A.), and later solubilized in 10 ml of Scintiverse® II scintillation fluid (Fisher Scientific, Fair Lawn, NJ, U.S.A.). Soluble [^{3}H]fucose was determined in the retinal samples by scintillation counting of a portion (1.0 ml) of the TCA/PTA supernatant in Scintiverse II. All scintillation counting was done on a Beckman LS 7000 scintillation counter. Maximum efficiency of the ^{3}H channel was 32% for the TCA/PTA supernatants and 21% for the TCA/PTA precipitates. All cpm were corrected for quenching according to quench standards and converted to dpm. The amount of TCA/PTA-precipitable radioactivity in each tissue sample of the uninjected side [right(contralateral) retina and optic nerve, and left (ipsilateral) optic tract, lateral geniculate body, and superior colliculus] was subtracted from the TCA/PTA-precipitable radioactivity present in each tissue sample of the injected side to correct for blood-borne labeling.

RESULTS AND DISCUSSION

The body temperature of the Chloropent-treated animals kept in an ambient temperature of 22.8°C decreased to 34.0 ± 0.7°C (mean ± SEM), whereas the body temperature (38.2 ± 0.2°C) of animals kept in an ambient temperature of 31.6°C was equal to that of the saline-treated animals (Fig. 1). The body temperatures of all three groups of animals were relatively stable during the axonal transport phase of the experiment.

Shuster and Hannam (1964) reported a decrease in brain protein synthesis in addition to an increase in the soluble amino acid pool in hypothermic (28–31°C) mice. We also found a trend toward more soluble [^{3}H]fucose present in the retinas of the hypothermic animals than in the 38°C animals (either saline- or Chloropent-treated). However, comparisons of the incorporation of [^{3}H]fucose into TCA/PTA-insoluble material in the retina revealed no significant differences in total dpm incorporated/μCi injected among the three experimental groups. The absence of temperature-related decreases in retinal fucosylation in the present experiment may be due to less hypothermia produced in the rats than that produced in the mice by Shuster and Hannam, or the fact that those investigators measured whole brain protein synthesis while we measured fucosylation of retinal proteins. Regardless of the source of this discrepancy, neither Chloropent treatment nor moderate hypothermia appeared to perturb the degree of fucosylation of retinal macromolecules for the time interval of the present experiment.

Figure 2 shows the relationship for each animal between body temperature and the amount of labeled glycoprotein transported to four structures of the retinofugal tract: optic nerve, optic tract, lateral geniculate body, and superior colliculus. Decreases in rapid glycoprotein transport did not correlate with Chloropent treatment as there were no differences ($p \geq 0.05$, Student's t test) in the amount of transported glycoproteins in the 38°C animals, re-

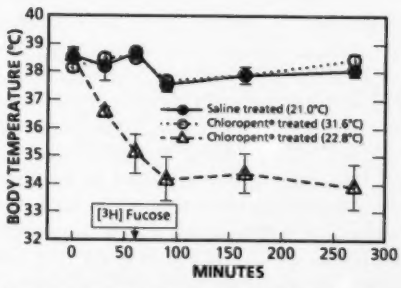

FIG. 1. Time course of deep (9–10 cm) rectal temperature of saline and Chloropent-treated rats (mean ± SEM). The mean ambient temperature for each of the three groups of animals is indicated in parentheses after each group label. The first saline or Chloropent injection was administered at 0 min, [^{3}H]fucose was injected intraocularly at 60 min, and a booster injection of saline or Chloropent was given at 90 min. All animals were killed at 270 min.

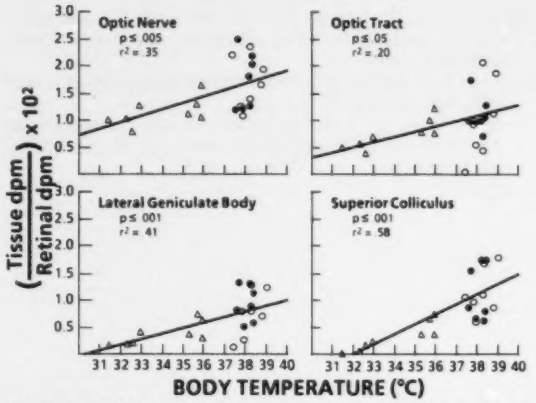

FIG. 2. Correlation of body temperature of individual rats and the amount of [³H]-glycoprotein transported to regions of the retinofugal tract of saline-treated (●); normothermic, Chloropent-treated (○); and hypothermic, Chloropent-treated animals (△). The vertical axis represents the TCA/PTA-insoluble dpm per tissue piece divided by the TCA/PTA-insoluble dpm in the corresponding retina to correct for possible differences due to injection and/or incorporation variability.

gardless of whether they were treated with Chloropent or saline. Rapid glycoprotein transport was, however, highly body temperature-dependent. Regression of body temperature versus the amount of transported glycoprotein in each structure produced lines with statistically significant positive slopes (p values are noted in Fig. 2), indicating that axonal transport decreased as a function of decreasing body temperature. The temperature dependence of rapid transport appeared to be magnified in the most distal structure, the superior colliculus, as illustrated by the significantly greater slope of the regression line for this structure than those lines calculated for the more proximal structures.

Previous axonal transport studies in poikilotherms have noted that decreases in the amount of transported material in the colder animals may be due either to a decrease in the total material transported or to an increase in latency before the material destined for transport leaves the cell body (Elam and Agranoff, 1971; Gross, 1973). Transport parameters determined over a course of several hours or days would be needed to distinguish between these two possibilities.

In summary, the present experiment showed that body temperature had a significant influence on the in vivo measurement of rapid axonal transport of glycoproteins in the rat optic system. This body temperature-dependent decrease in transport, depending on the distance from the perikaryon, could be significant in animals whose body temperature was only 2°C below normal.

This finding should be taken as a cautionary note for those investigators exploring perturbations of axonal transport, especially rapid transport, in animals whose thermoregulatory physiology may be disrupted. Many compounds or metabolic conditions may render mammals poikilothermic. Even slight decreases in body temperature may be responsible (rather than the primary treatment) for reductions in axonal transport.

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